ENHANCED DISSOLVED ORGANIC MATTER RECOVERY FROM SALTWATER SAMPLES WITH COUPLED ELECTRODIALYSIS AND SOLID PHASE EXTRACTION

A Thesis
Presented to
The Academic Faculty

by

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In Partial Fulfillment
of the Requirements for the Degree
Master of Science in the
School of Earth and Atmospheric Sciences

Georgia Institute of Technology
December 2015

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Date Approved: November 30, 2015

ACKNOWLEDGEMENTS

I would like to thank my labmates, advisor, and other members of the EAS faculty for providing me with direction and encouragement. Special thanks go to the wonderful people at the Skidaway Institute of Oceanography the crew members of the R/V Savannah for the support and fun times.

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LIST OF SYMBOLS AND ABBREVIATIONS

DOM	Dissolved Organic Matter
ED	Electrodialysis
SPE	Solid Phase Extraction
PPL	Styrene-divinylbenzene Bond Elut PPL Resin
EEM	Excitation Emission Matrices
DOC	Dissolved Organic Carbon
RO	Reverse Osmosis
TN	Total Dissolved Nitrogen
LCD	Limiting Current Density
µS	Micro-Siemens
mS	Milli-Siemens
TOC	Total Organic Carbon
GF/F	Glass Fiber Filter
EDTA	Ethylenediaminetetraacetic Acid
C/N	Ratio of Caron to Nitrogen
DON	Dissolved Organic Nitrogen
DIN	Dissolved Inorganic Nitrogen
µM	Micro-Molar
FDOM	Fluorescent Dissolved Organic Carbon

SUMMARY

Complexities associated with dissolved organic matter (DOM) isolation from seawater have hampered compositional characterization of this key component of global carbon and nutrient cycles. Two techniques, Electrodialysis (ED) and Solid Phase Extraction (SPE), were combined to more effectively isolate DOM from salt-containing waters. Sample recovery was optimized and evaluated on a range of samples including coastal ocean seawater, open ocean seawater, artificial seawater from cultures of marine phytoplankton, and artificial seawater samples containing standard compounds of different molecular sizes and charge. ED was performed with a system optimized for processing 2 to 10 L sample volumes and SPE was performed using Bond Elut PPL exchange resin. With the combination of ED and PPL techniques an average recovery of 76.7 ± 2.6% was obtained for natural coastal seawater. Comparison of C/N ratios and fluorescence excitation emission matrices (EEMs) taken at the beginning and end of the recovery process indicated that the final recovered material was representative of the DOM present in the original samples.

CHAPTER 1

INTRODUCTION

Marine dissolved organic matter (DOM) represents one of the world's largest reservoirs of exchangeable carbon (Hansell and Carlson, 2015). Most chemical and spectral characterizations of DOM in seawater are confounded by the high inorganic salt concentrations (~35 g/L) and comparatively low DOM concentrations (~0.5 mg/L) (Mopper et al., 2007). Therefore, DOM must be concentrated and isolated before most detailed compositional characterizations can proceed (Repeta, 2015). Over the past few decades many technologies have been developed to isolate DOM from marine samples.

Solid-phase extraction (SPE) using polystyrene or octadecyl-silica (C-18) resins was one of the first viable methods for isolating DOM from seawater. The availability of SPE sorbents has grown to include cross-linked polystyrene (XAD-2, -4, and -16) and their derivatives (PPL), Isolute ENV, and polyacrylate (XAD-8) (Mopper et al., 2007; Repeta, 2015). Overall recovery efficiencies and selectivity toward different molecular classes differ among SPE sorbents. Because of this selectivity, samples are sometimes processed with more than one SPE sorbent in series to maximize recovery (Green et al., 2014). Some of the highest DOM recoveries have been achieved with PPL resins with up to 62 ± 6% recovery of dissolved organic carbon (DOC) in brackish waters and approximately 43 ± 5% for purely marine DOC (Dittmar et al., 2008). When processing with SPE, filtered samples are typically acidified with hydrochloric acid to pH 2-2.5 and eluted with pure methanol. Acidification and methanol treatments could create unknown effects on the sample composition (Repeta, 2015). Another method adopted for DOM

isolation is tangential flow ultrafiltration, which avoids the chemical treatments and selective nature of SPE resins, only recovers the high molecular weight fraction of DOM. Recovery of DOM with tangential flow ultrafiltration is highly dependent on the membrane material and sample processing protocols. With tangential flow ultrafiltration up to 30% of the DOC can be retained but final recoveries are often subject to losses associated with high concentration factors and sample desalting during diafiltration (Repeta, 2015). This leads to typical DOC recoveries of 10-15% with ultrafiltration (Repeta, 2015).

Advances in DOM isolation have been achieved through the coupling of reverse osmosis (RO) and electrodialysis (ED) (Diaz et al., 2012; Gurtler et al., 2008; Koprivnjak et al., 2006; Vetter et al., 2007; Young and Ingall, 2010). In this method, salt is removed from a sample by ED utilizing an alternating series of positive and negative ion-exchange membranes under the influence of an electric potential, while simultaneously reducing sample volume by RO (Repeta, 2015). Reported ED/RO DOM recoveries range from 50% to > 100% with an average carbon recovery of 76% (n = 21) (Repeta, 2015). It should be noted that the recovered fraction claimed in many of these studies includes a fraction desorbed in a strong base (pH = 12) rinse of the system after sample processing (Green et al., 2014). This fraction, although technically recovered, is not typically analyzed because the presence of strong base confounds characterization methods and likely alters DOM composition. Without inclusion of the base rinse fraction, recoveries by ED/RO are approximately 16% lower (Green et al., 2014). Most ED/RO systems built to isolate DOM from natural seawater samples are designed for large (approximately 200 L) sample volumes in order to obtain sufficient DOM mass for chemical analyses. This

makes the systems physically large, and therefore unruly to manage. Such systems typically require a minimum volume of approximately 3 L to prevent pump cavitation, which ultimately limits sample concentration by the RO side of the system. In large ED/RO systems sample is processed down to 3-5 L of low salinity water, which is typically freeze-dried to further concentrate the DOM.

For this study, a table-top ED system was designed and constructed to process 2-10 L samples from a salinity of approximately 35,000 ppt to approximately 50 ppt in 3-5 hours. This system was designed to be small and maneuverable, with the entire system occupying less than 1 m^3 and having a minimum operating volume of 200 mL. With this system the 2-10 L starting volume is directly freeze-dried after processing, removing the need for water volume reduction by RO. Thus, DOM losses that occur during RO processing are avoided. DOM retention during sample processing was evaluated using coastal ocean seawater, open ocean seawater, artificial seawater from cultures of marine phytoplankton, and artificial seawater samples containing standard compounds of different molecular sizes and charge. Additionally, various operational parameters were tested and optimized to improve recoveries. Initial experiments with the system indicated that the DOM molecular classes with lower recoveries in ED could be effectively recovered by PPL. PPL sorbents are made for the extraction of a wide range of highly polar to nonpolar substances (Dittmar et al., 2008) and may capture the molecule classes missed by ED. Hence, the two techniques were coupled to improve total DOM recoveries and to recover a more representative fraction of the molecular classes present in the original DOM sample.

CHAPTER 2

EXPERIMENTAL MATERIALS AND METHODS

Electrodialysis

Electrodialysis was performed using Deukum electrodialysis stack (Deukum GmbH, Frickenhausen, Germany) coupled to circulation system built in our lab. The circulation system is composed of three pumps and associated adjustable valves used to control the flow and pressure of the sample (diluate), concentrate (water flow that receives ions from the sample), and electrode rinse (flow that maintains ionic balance between the cathode and anode). In addition to the pumps, the system includes an Oakton Cond 6+ conductivity meter with the electrode mounted in a laboratory-built housing so it could be used inline, and three high-density polyethylene (HDPE) tanks to store the diluate, concentrate, and electrode rinse. A 10 cell pair electrodialysis stack was constructed consisting of alternating Neosepta anion AMX (strongly basic, 2.0–3.5 Ω cm^2 at 25ºC) and Neosepta cation CMX (strongly acidic, 1.8–3.8 Ω cm^2 at 25 ºC) exchange membranes. These membranes are held between opposing flow regulating end blocks of the stack, which respectively house a platinized titanium mesh anode and a stainless steel cathode. CMX membranes were doubled up at the side of the stack closest the cathode (Pfromm et al., 1999). The membranes are separated by 0.7 mm thick flexible turbulence promoting spacers and have a cross-sectional active membrane area of approximately 100 cm^2. The electric potential across the stack was supplied by a 1.2 kW Sorensen DCS150-8EM1 solid-state power supply. During the ED process anions are pulled through the AMX membranes toward the anode, while cations are pulled through

CMX membranes toward the cathode. As the ions are pulled to their respective electrodes they move out of the diluate flow channel and into the concentrate flow channel.

After numerous optimization trials, the following procedure was developed for cleaning and preparing the ED system. An electrode rinse solution is made by dissolving 60 g of Sodium Sulfate (Na_2SO_4) into 2 L of Milli-Q water (Pfromm et al., 1999). The electrode rinse tank is filled with the electrode rinse solution and the concentrate tank filled with 6 gallons of Milli-Q water. With all three channels in circulation, the diluate channel is rinsed 6 times with 1 L of Milli-Q water for 2 minutes per rinse. After the diluate channel rinses, the concentrate solution is replaced with 6 gallons of fresh Milli-Q water. Before sample processing the diluate channel is rinsed with two 300 mL sample aliquots for 2 minutes per rinse. The pH of the electrode rinse should be monitored before and after a sample is processed. If the pH falls outside the range of 5.5-7, the electrode rinse flow channel should be rinsed three times with Milli-Q water and the electrode rinse replaced. A pressure of 4.5 psig was maintained in all flow channels during cleaning and sample processing to prevent pressure differences across the membranes.

The following procedure based on numerous tests and optimizations was developed for ED processing of samples. A sample is placed in the diluate tank and circulated through the system for 2 minutes to ensure good mixing before the electricity is supplied. At this point the conductivity of the sample is measured and a 40 mL subsample is collected for eventual DOC and total dissolved nitrogen (TN) analysis (subsample $T_{initial}$). Once the power is supplied to the system, it is vital that the both the conductivity of the sample and the applied amperage are continuously monitored to keep the system below the limiting current density. If the electrical current exceeds the limiting

current density (LCD) ion concentrations drop to near zero at the membrane surface on the diluate side of the membranes (Pfromm et al., 1999). The absence of ions at the membrane surface results in the splitting of water molecules. The hydroxyl ions resulting from the splitting of water pass through the membranes causing a rapid increase in the pH of the concentrate and a corresponding decrease in the pH of diluate (Pfromm et al., 1999).

During sample processing the conductivity of the concentrate and sample is continuously measured. When the conductivity of the concentrate exceeds that of the sample by approximately 2 mS the system is briefly shut off and the concentrate is changed. Optimization experiments indicate that sample DOM recoveries are highest when one gallon of concentrate is left in the tank and 5 gallons of the concentrate replaced with Milli-Q water rather than completely replacing the concentrate with Milli-Q water. After a concentrate change, the system is turned back on and the sample is processed to the desired end point, typically a conductivity of approximately 150 μS. At the end of the ED process a 40 mL subsample is collected (subsample T_{final}) to calculate the total DOC and TN recoveries. It should be noted that near the end of the process when sample conductivities are less than 400 μS, the limiting current density is difficult to define. At this point samples were run at a current of 0.13 amps until the final target conductivity was reached. At these final stages of sample processing, it was found that reducing the electric current below 0.13 amps led to impractically long run times, while not benefitting the recovery. At the end of a run, the diluate channel is cleaned by rinsing 6 times with 1 L of Milli-Q water for 2 minute per rinse. If the membrane stack is not

going to be used to process samples within one day, an approximately 100 mS NaCl brine solution is run through the electrodialysis stack to hinder biological growth in the system.

PPL

Bond Elut PPL, manufactured by Agilent Technologies, is a styrene-divinylbenzene (SDVB) polymer that has been modified with a proprietary non-polar surface. The protocol for DOM extraction via SPE using PPL sorbent was performed according to an adjusted scheme based previous work (Dittmar et al., 2008), and the manufacturer's guidelines using 6 mL Bond Elute PPL cartridges (columns).

Before DOM can be extracted with PPL columns the sorbent must first be conditioned and equilibrated. This is a two-day process with several cycles of soaking and rinsing the sorbent. On the first day the cartridge is filled and drained three times with methanol (LC/MS Grade), then immediately rinsed with three cartridge volumes of Milli-Q water, and again rinsed with three cartridge volumes of methanol. The cartridge is then filled with methanol and left over night (minimum of four hours). On the second day of conditioning, a pH 2 Milli-Q solution is made by adding 1 mL of 12 N HCl (ACS grade or higher; 32%) to 1 L of Milli-Q water. The cartridge is then rinsed three times with a cartridge volume of Milli-Q water, followed by three cartridge volumes of methanol, and finally three cartridge volumes of the pH 2 Milli-Q solution. The extraction step was immediately preformed after the cartridge final rinse.

Prior to PPL processing samples are acidified to pH 2 with HCl (ACS grade; 32%). Acid washed (pH 2 Milli-Q water) Teflon tubing (ID 2 mm), Luer adaptors, and fittings were attached to the cartridges and used to siphon the sample through the PPL. The flow was adjusted to approximately 10 mL per minute. Initial and final weights of

the samples were recorded to determine the total processed volumes. Once the entire sample has passed through the column, the PPL columns were treated with three cartridge volumes of pH 2 Milli-Q water. Zero-grade air is then passed through the cartridge at approximately 10 psig in an effort to dry the resin before elution. Once the columns are dry, DOM sorbed to the column is eluted with approximately 9 mL of methanol. The volumes of the eluted methanol were measured gravimetrically. Eluted samples were stored at -20 ^{o}C until processing.

To calculate final DOM recoveries, a precisely measured aliquot of the methanol extract is placed in a vented drying oven at 40 ^{o}C for 24 hours to dry the sample. The sample is then resuspended in 20 mL of pH 2 Milli-Q water and sonicated for 10 minutes. Concentrations of DOC and TN, were then determined using a Shimadzu TOC-VCSN total organic carbon (TOC) analyzer (see below).

Combined PPL – ED Natural Samples

DOC recovery using the combined PPL/ED technique was evaluated using saltwater samples collected between the dates of 09/03/2014 and 12/10/2014 from the Skidaway River (31°59'24.1"N, 81°01'20.9"W) off the main dock of the Skidaway Institute of Oceanography located near Savannah Georgia, U.S.A. The samples were collected 1 m below the water surface at approximately one hour after high tide to obtain waters when they are at their maximum salinity during a tidal cycle. The samples, which had initial conductivities between 38-47 mS, were filtered through an acid cleaned Whatman Polycap TC Filter Capsule with pore size 0.2 µm and stored in acid washed Nalgene 20 L carboys at 4 ^{o}C before processing.

Seven samples were collected on different days throughout September 2014 and processed using ED within three days of collection. Two approximately 25 L samples were collected on 10/21/14 and 11/25/14 and placed in cold storage in acid washed carboys. Two liter aliquots of the sample collected on 10/21/14 and were processed in triplicate on ED within approximately one month of the collection date. One 2 L aliquot of the sample collected on 10/21/14 was processed with first processed with PPL and subsequently processed with ED. Six 2 L aliquots were taken from the sample collected on 11/25/14. Three of these aliquots were processed in triplicate solely using ED and three were processed in triplicate with PPL and subsequently processed with ED. All of the aliquots from the sample collected on 11/25/14 were processed within two weeks of the collection date. Note that the samples processed with PPL then ED were acidified to pH 2 before processing on PPL and not neutralized before ED processing.

Standards and Samples for ED Optimization

Samples of artificial seawater from phytoplankton cultures and open ocean seawater (described below) were processed with ED to evaluate and optimize the DOM recovery of samples presumably representing freshly produced and extensively cycled DOM, respectively. Additionally, to evaluate the recovery of specific molecular classes, three standard reagent grade compounds, including D-(+)-Glucose ($C_6H_{12}O_6$; Sigma-Aldrich ≥99.5%) Ethylenediaminetetraacetic acid (EDTA; $(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$; Sigma-Aldrich, ACS grade), and Vitamin B_{12} ($C_{63}H_{88}CoN_{14}O_{14}P$; Sigma-Aldrich, ≥98%), were each individually dissolved in artificial seawater (Grasshoff et al., 1999) and processed with ED.

Two species of marine phytoplankton obtained from the Bigelow National Center for Marine Algae and Microbiota (NCMA) were grown in axenic laboratory cultures and processed with ED. *Thalassiosira pseudonana* (CCMP 1335), a diatom was cultured with Si replete $^{f}/_{2}$ media (ncma.bigelow.org/media/pdf/NCMA-algal-medium-f_2.pdf) under axenic conditions in artificial seawater. The biomass of *Thalassiosira pseudonana* was harvested via centrifugation at 4500 rpm for 5 minutes and the supernatant containing DOM exudates was filtered through a glass fiber filter (Whatman 1825-150 GF/F, pore size 0.7 μm) before processing with ED. *Emiliania huxleyi* (CCMP 371), a coccolithophore, was cultured with $^{L1}/_{25}$ media (ncma.bigelow.org/media/pdf/NCMA-algal-medium-L1.pdf) under axenic conditions in artificial sea water. The particulate biomass of *Emiliania huxleyi* was separated from the cultures using a polycarbonate filter (pore size 0.2 μm) and the filtrate containing DOM exudates was frozen until ED processing. These phytoplankton were cultured under a spectrum of nutrient conditions as part of a different study.

Open ocean seawater samples were collected using the underway pumping system on the R/V Savannah in Atlantic Ocean waters located approximately 60 miles east of Savannah, Georgia on 1/13/14. The samples were filtered through an acid cleaned Whatman Polycap TC Filter Capsule (0.2 μm) and stored in several 20 L acid washed Nalgene carboys at 4 ^{o}C before processing with the ED. Two liter aliquots of this sample were processed with ED under a spectrum of running conditions in an effort to optimize the processing protocol for time, recovery, and ease of use. Once a final protocol was determined triplicate aliquots of the underway sample and all other samples in this study were processed in accordance with the final protocol described above.

Sample Analysis

DOC concentrations were used to monitor DOM recovery during sample processing. DOC concentrations in subsamples (40 mL), were measured as non-purgeable organic carbon using high temperature catalytic oxidation with a Shimadzu TOC-VCSN or TOC-VCPH analyzer (Grasshoff et al., 1999). DOC recoveries were calculated by dividing the mass of DOC at the end of processing by the mass of DOC initially supplied. Calculated recoveries also account for the removal of DOC in subsamples taken for measurements. The Shimadzu instruments above were also used to measure TN concentrations in the subsamples. During ED processing >99% of the dissolved inorganic nitrogen (DIN) is removed from the sample. Thus, TN concentrations determined at the end of the ED process for all sample types represent DON concentrations. To calculate initial DON concentrations in seawater and cultured phytoplankton samples, DIN concentrations must be subtracted from TN concentrations. For seawater samples collected at the Skidaway dock, DIN was estimated using average annual NO_3^- and NH_4^+ concentrations of 2.2 µM and 2.7 µM, respectively, for the Skidaway River estuary (Verity, 2002). Initial DIN concentrations for cultured phytoplankton filtrates were measured as ΣNOx using anion chromatography (Metrohm A Supp 5 150.0/x 4.0 mm) coupled to ultraviolet detection (Beckman Coulter DU 720 detector) with a NaCl eluent (Beckler et al., 2014). Initial and final carbon to nitrogen (C/N) ratios were calculated by dividing the DOC concentrations by the dissolved organic nitrogen (DON).

EEM spectra for Skidaway dock seawater samples were measured on subsamples collected during ED processing in 1 cm quartz cells in a Horiba AquaLog VS140 (CCD1)

spectrofluorometer. EEMs are produced from multiple emission spectra collected at successively increasing excitation wavelengths (Fellman et al., 2010; Stedmon and Bro, 2008). EEMs can be used to categorize a subset of colored dissolved organic matter known as fluorescent dissolved organic matter (FDOM) (Stedmon and Álvarez-Salgado, 2011; Stubbins et al., 2014). The peaks present on an EEM surface correlate to different molecular classes within the sample (Coble et al., 1998; Coble et al., 1990; Fellman et al., 2010; Jaffe et al., 2014). Florescence and absorbance measurements for EEMs were processed in similar fashion to previous studies (Coble et al., 1998; Coble et al., 1990; Fellman et al., 2010; Stubbins et al., 2014). However, the Horiba Aqualog and its included software allowed for simultaneous data collection and EEM generation. A Milli-Q water blank EEM was subtracted from each sample EEM to remove Raman scatter peaks (Coble et al., 1998). Rayleigh scattering was removed from EEMs by smoothing with surrounding emission values.

CHAPTER 3

RESULTS AND DISCUSSION

Results

For all thirteen seawater samples collected at the Skidaway dock, DOC recoveries averaged 71.3 ± 6.5% using ED (Figure 1). Two seawater samples collected at the Skidaway dock were divided into three aliquots, with each aliquot processed separately through ED to evaluate reproducibility of DOC recovery. Recovery was 71.1 ± 4.8% for the sample collected on 11/25/2014 and 69.8 ± 11.6 for the sample collected on 10/21/2014. The absence of obvious trends in DOC recovery related to length of sample storage before processing (Figure 1) suggests that storage of up to one month at 4 ^{o}C is acceptable.

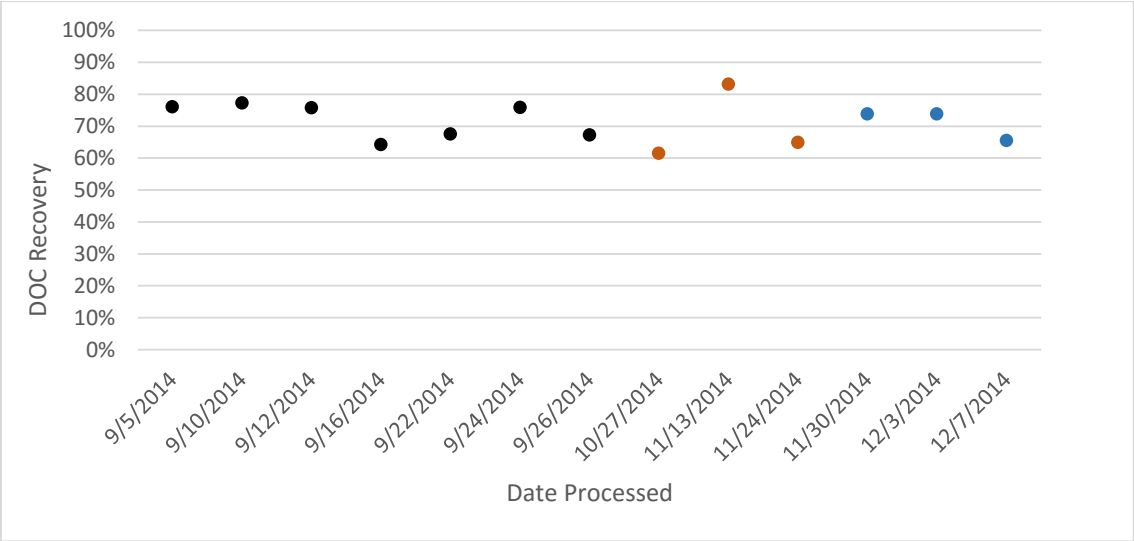

Figure 1 DOC recoveries of Skidaway dock seawater samples using ED only. Black points are for individual samples collected September 2014. Blue and orange points are for triplicate analysis of samples collected on 11/25/2014 and 10/21/2014 respectively.

Open ocean samples collected off the coast of Savannah and processed in triplicate with ED had recoveries of 50.5 ± 3.1%. The average DOC recovery using ED for all cultured phytoplankton filtrates, *Emiliania huxleyi* and *Thalassiosira Pseudonana*, averaged 71.0 ± 12.5% (Figure 2). Glucose, EDTA, and vitamin B_{12} were processed with ED in triplicate and had respective DOC recoveries of 90.2 ± 2.1%, 67.5 ± 9.9%, and 98.3 ± 1.6%.

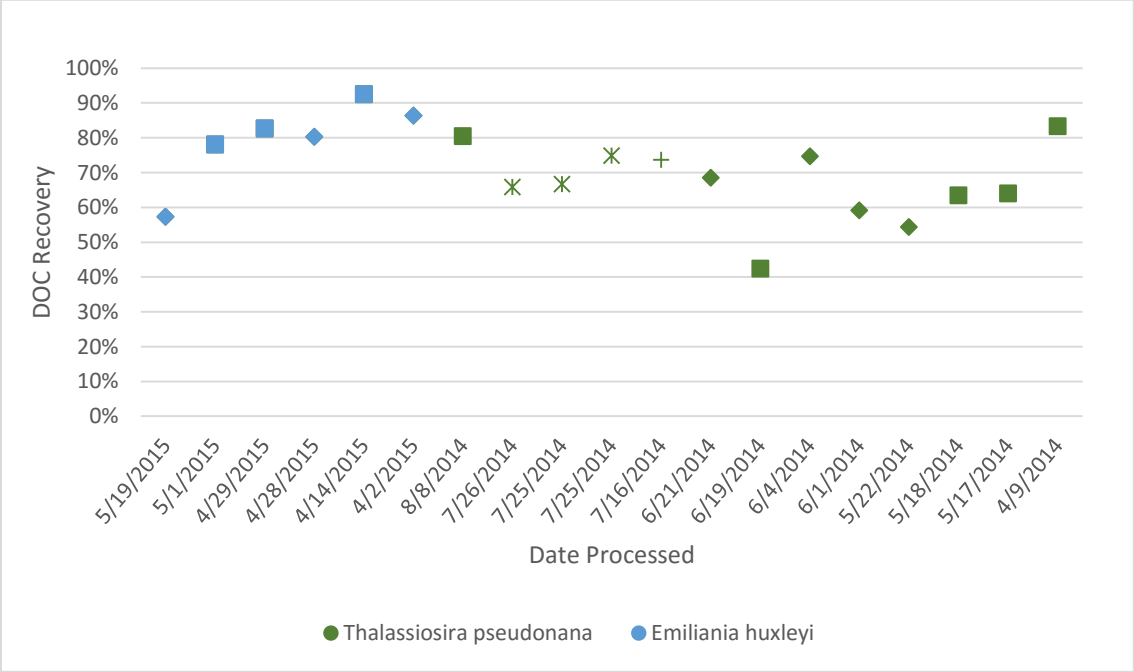

Figure 2 DOC recoveries of phytoplankton filtrates cultured under different growth conditions. Diamonds represent P-stressed cultures, squares represent P-replete cultures, asterisks represent N-stressed cultures, and plus signs represent P+N replete cultures.

The average DOC recovery using solely PPL on four Skidaway dock seawater samples was 38.1 ± 2.3% (Figure 4). The combined PPL/ED process on these samples achieved an average DOC recovery of 76.7 ± 2.6% (Figure 3).

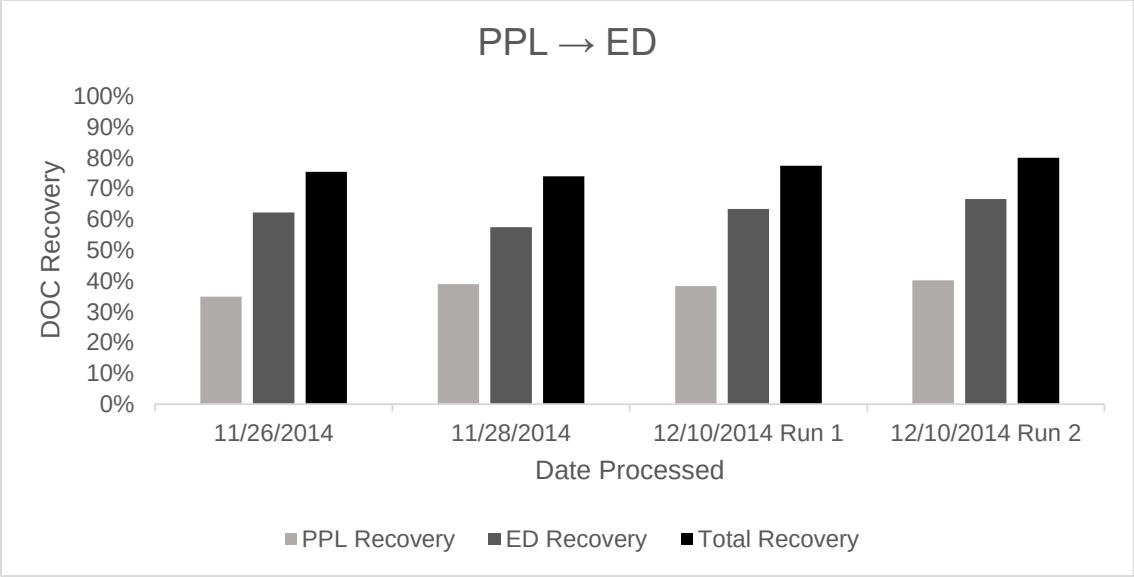

Figure 3 DOC recoveries for Skidaway dock seawater samples processed with PPL then ED.

Initial C/N ratios at the start of the ED process averaged 19.3 ± 3.6 for Skidaway dock seawater samples and final C/N ratios for these samples at the process end point were 18.9 ± 1.5, excluding one sample taken on 12/7/2014 (Figure 4). Other than the 12/7/2014 sample, DOM C/N initial and final ratios are on average within 12.1% of one another (Figure 4). Initial and final C/N ratios calculated for *Emiliania huxleyi* are on average within 2.5% of each other (Table 1). Note TN was not measured on *Thalassiosira Pseudonana* filtrates. Initial and final C/N ratios calculated for the EDTA and vitamin B_{12} standards are on average within 4.8% of each other (Table 2). EEMs for the initial and final subsamples collected during ED processing have peaks with the same proportional intensities that are situated in the same region on the spectral surface (Figure 5).

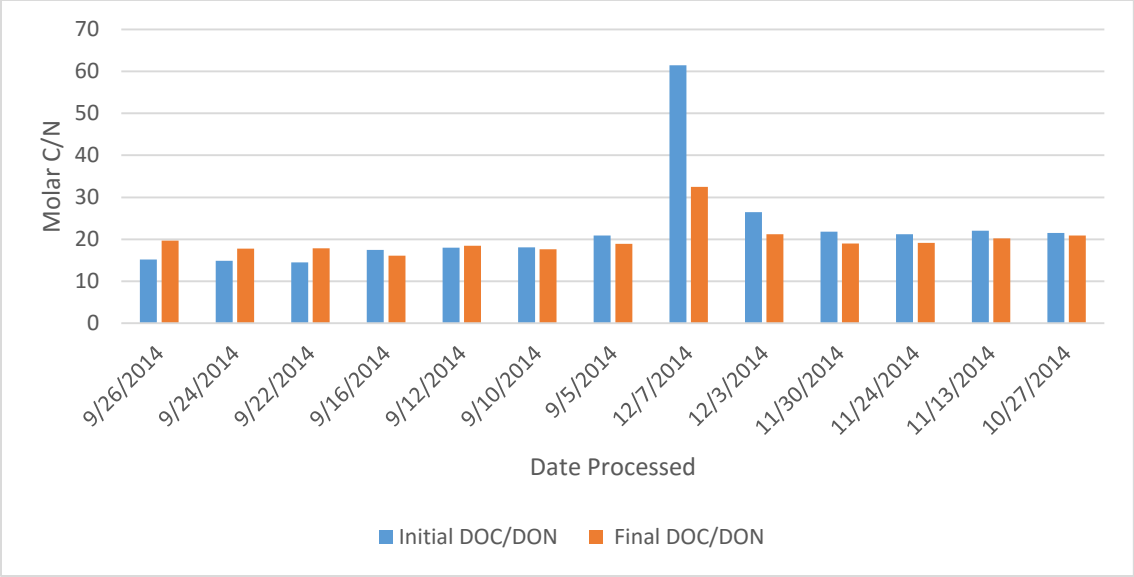

Figure 4 Molar C/N ratios of Skidaway dock seawater samples.

Table 1 Molar C/N ratios for *Emiliania huxleyi.*

Date Processed	DOC Recovery (%)	Initial C/N	Final C/N	Experiment
5/19/2015	57.3	9.2	8.0	P stressed A
4/28/2015	80.3	3.5	3.1	P stressed B
4/2/2015	86.3	3.2	3.5	P stressed C
4/29/2015	82.7	5.9	5.3	P Replete B

Table 2 Molar C/N ratios of standard compounds.

Substance	DOC Recovery (%)	Initial C/N	Final C/N
B12	100.0	3.9	4.0
B12	97.0	4.1	4.1
B12	97.9	4.1	4.1
EDTA	70.4	4.6	5.0
EDTA	75.6	4.7	5.2
EDTA	56.3	4.8	5.2
Glucose	92.7	-	-
Glucose	89.3	-	-
Glucose	88.7	-	-

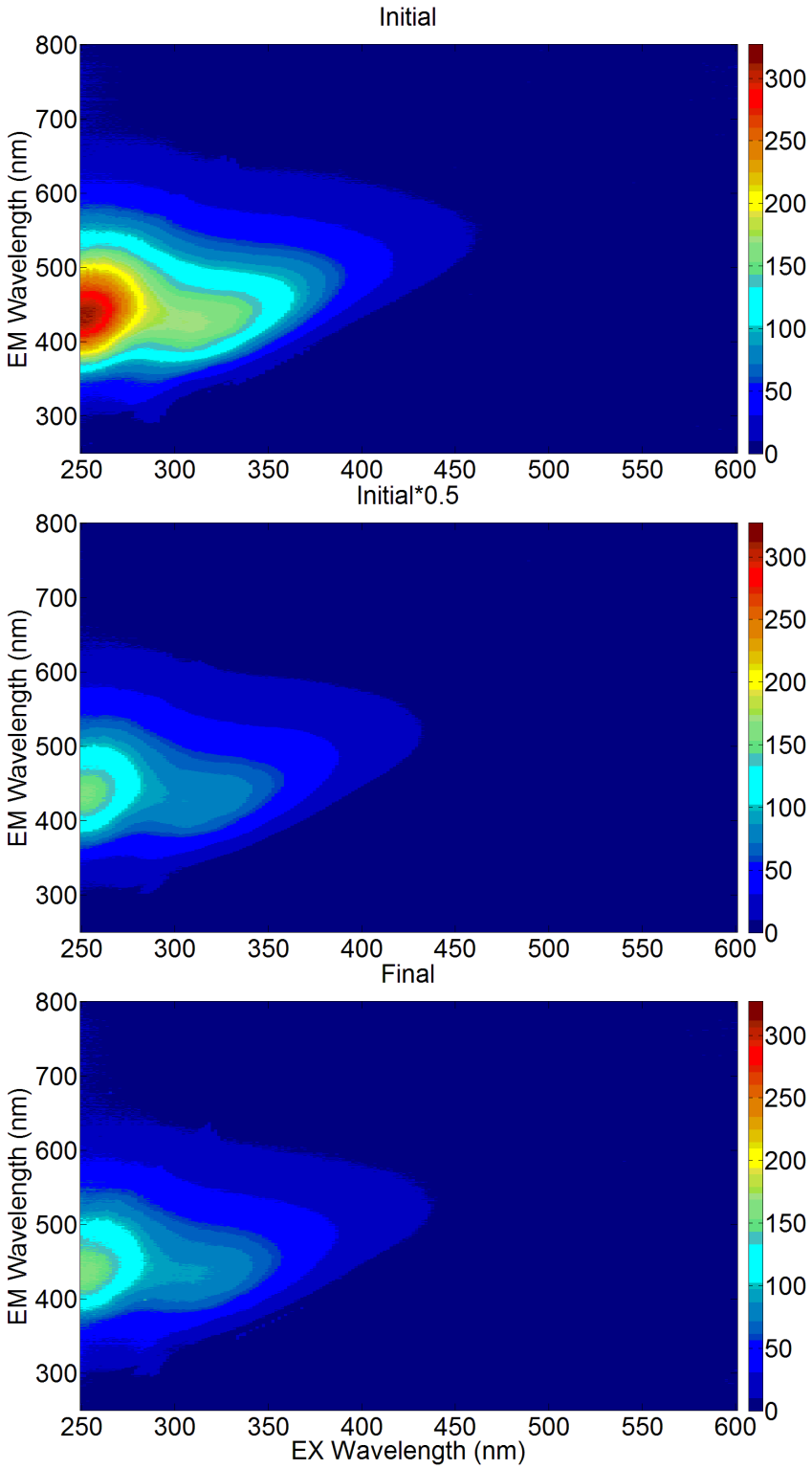

Figure 3 The top EEM is composite average spectra for the initial ED subsamples for all Skidaway dock seawater samples processed on the ED and the bottom EEM shows the same for the final subsamples. The fluorescence intensities for the final ED subsamples are approximately 50% of the initial subsamples. The middle EEM shows the initial EEM with the fluorescence intensities halved for comparison.

Discussion

Samples processed solely with ED achieved high DOC recoveries for all sample types; Skidaway dock seawater 71.3 ± 6.5%, open ocean 50.5 ± 3.1%, phytoplankton cultures 71.0 ± 12.5%, EDTA 67.5 ± 9.9%, Glucose 90.2 ± 2.1%, and B_{12} 98.3 ± 1.6%. These recoveries clearly exceed the typical DOC recovery of tangential flow ultrafiltration of approximately of 10-15% (Repeta, 2015). These recoveries are also generally higher than those achieved using solely PPL resins. DOC recoveries using PPL appear to be quite variable depending on sampling location, indicating a potential influence of the organic composition on DOC recovery. High values of DOC recovery using PPL seem to be achieved in some sampling locations that have a strong terrestrial influence on DOC composition. For example, PPL recoveries of 65 ± 6% and 62 ± 6% have been obtained for Apalachicola salt marshes and the North Brazil shelf, respectively (Dittmar et al., 2008). High DOC recoveries with PPL for the Skidaway dock seawater samples were not obtained despite their collection in an estuarine system with high terrestrial DOC inputs. Recoveries using PPL only for the seawater samples collected at the Skidaway main dock of 38.1 ± 2.3% were similar to those of open ocean studies in the Gulf of Mexico and the Weddell Sea of 43 ± 2% and 43 ± 5%, respectively (Dittmar et al., 2008). Lower recoveries obtained for Skidaway dock seawater samples in comparison to other estuarine systems may be attributed to sample collection around high tide, which would increase the relative contribution of marine DOC to the sampling site.

Recoveries in this study using ED only are in the same range as the DOC recoveries reported for combined ED and RO (ED/RO) of 50% to > 100% (Repeta, 2015). The recoveries of >100% in the previous study may result from carbon desorption

from the large membrane surface areas required by these systems to process approximately 200 L sample volumes. The average DOC recovery of 76% (n = 21) by ED/RO (Repeta, 2015) includes an up to 16% fraction desorbed in a strong base (pH =12) rinse of the system after sample processing (Green et al., 2014). This base fraction is not typically analyzed due to the potential for compositional modifications of DOM at high pH and analytical challenges of introducing this solution into sensitive instrumentation. Without inclusion of the base rinse fraction, recoveries by ED/RO would be better characterized as approximately 60%. The small volume design of the system used in this study eliminates the need for RO to reduce sample volumes for subsequent freeze-drying. Elimination of RO processing also greatly reduces the membrane surface area contacted by the sample, which increases overall recovery and eliminates the need for a base rinse of the system to desorb DOM from RO membranes.

DOC recovery using ED only was assessed for different organic matter types by examining filtrates from cultures of two common marine phytoplankton. In contrast to ambient marine DOM, the DOM produced in these axenic phytoplankton cultures is not modified by microbial decomposition and would be expected to contain a high proportion of freshly produced labile molecules that are not typically present in natural sea water samples. Comparison of the Skidaway dock seawater samples with an average DOC recovery of 71.3 ± 6.5% to the phytoplankton cultures with average DOC recoveries of 71.0 ± 12.5% suggests that differing proportions of freshly produced DOM in a sample does not strongly influence overall DOC recoveries.

A 90.2 ± 2.1% recovery of glucose (molecular weight 180.2) was achieved using ED. In contrast, such low molecular weight molecules will typically pass through

membranes in isolation techniques such as ultrafiltration resulting in extremely poor recoveries (Repeta, 2015). EDTA, another low molecular weight molecule (molecular weight 292.2), has a charge of -4 at the ED operating pH. The lower recovery of EDTA (67.5 ± 9.9%) relative to glucose likely results from attractive forces pulling negatively charged EDTA through the membrane towards the anode, reducing recovery. The difference between glucose and EDTA recoveries suggests that charge plays a larger role than size for molecular retention during ED. Vitamin B_{12} is a larger molecule (molecular weight 1355.4) than both glucose and EDTA but it is electrically neutral at the pH value maintained during ED processing. The size and charge characteristics of B_{12} are both likely factors in its high (98.3 ± 1.6%) recovery during ED. Although the recovery of EDTA is less than the other standard molecules tested, it is still far above typical recoveries obtained by other membrane based isolation techniques. ED recovery of standard compounds suggests some differences in recovery for certain molecular types, such as highly charged molecules.

High recoveries achieved on all sample types with ED leave less room for size and/or compositional selectivity during the DOM isolation process relative to previous methods. Reduced compositional and size selectivity during ED relative to other methods is reflected in the comparison of C/N and EEM analyses of initial and final subsamples. The percent difference between initial and final molar C/N ratios obtained using ED were small in all sample types; Skidaway dock seawater 12.1%, phytoplankton cultures 2.5%, standard compounds 4.8%. This suggests that ED isolation of DOM is not strongly selective in terms of the C/N ratio of molecules retained. The peaks on the initial and final EEMs are situated in the same regions and have the same proportional intensities

suggesting that ED is not selective toward the molecular classes present in the FDOM fraction characterized by this method.

Coupling PPL with ED leads to a higher average DOC recovery of 76.7 ± 2.6%. PPL pre-treatment removes approximately 38.1 ± 2.3% of the DOC in Skidaway dock seawater samples. Subsequent ED of the sample pre-treated with PPL achieves a 62.4 ± 3.8% DOC recovery (Figure 3). For Skidaway dock seawater samples, the sole use of PPL would recover only about half of the DOM. PPL processing adsorbs molecules that are reactive to the resin surface. Such surface-reactive molecules are potentially adsorbed to ED membranes and can therefore be lost during ED processing. Running samples through PPL as a pre-treatment before ED likely reduces the loss of surface-reactive molecules during ED by extracting them during PPL processing. There is an additional benefit to the removal of surface reactive molecules before ED. Such molecules can attach to the ED membranes and decrease ED efficiency by increasing electrical resistance across the membrane stack (Lindstrand et al., 2000). Previous studies have attempted to prevent organic molecule adsorption by pulsing the electricity supplied during ED (Gurtler et al., 2008; Ruiz et al., 2007). However, pulsing the electricity can increase processing times with minimal gains in overall recovery.

CHAPTER 4

CONCLUSIONS

ED processing in a system optimized for small volume samples (2-10 L) achieves high DOC recoveries in natural and artificial seawater samples; Skidaway dock seawater 71.3 ± 6.5%, open ocean 50.5 ± 3.1%, phytoplankton cultures 71.0 ± 12.5%. Sample processing takes 3-5 hours and small starting sample volumes alleviate the need for RO used by other methods to reduce sample volumes. An approximately 5% percent increase in DOC recovery can be obtained through sample pre-treatment with PPL before ED processing. Similar DOC recoveries of the Skidaway dock seawater samples and axenic phytoplankton culture artificial seawater samples suggests that differing proportions of freshly produced molecules does not influence recoveries. Initial and final C/N ratios as well as EEMs indicate the recovered DOM is compositionally representative of the DOM initially present in the samples.

REFERENCES

Beckler, J. S., Nuzzio, D. B., and Taillefert, M., 2014, Development of single-step liquid chromatography methods with ultraviolet detection for the measurement of inorganic anions in marine waters: Limnology and Oceanography-Methods, v. 12, p. 563-576.

Coble, P. G., Del Castillo, C. E., and Avril, B., 1998, Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon: Deep-Sea Research Part Ii-Topical Studies in Oceanography, v. 45, no. 10-11, p. 2195-2223.

Coble, P. G., Green, S. A., Blough, N. V., and Gagosian, R. B., 1990, CHARACTERIZATION OF DISSOLVED ORGANIC-MATTER IN THE BLACK-SEA BY FLUORESCENCE SPECTROSCOPY: Nature, v. 348, no. 6300, p. 432-435.

Diaz, J. M., Ingall, E. D., Snow, S. D., Benitez-Nelson, C. R., Taillefert, M., and Brandes, J. A., 2012, Potential role of inorganic polyphosphate in the cycling of phosphorus within the hypoxic water column of Effingham Inlet, British Columbia: Global Biogeochemical Cycles, v. 26, p. 13.

Dittmar, T., Koch, B., Hertkorn, N., and Kattner, G., 2008, A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater: Limnology and Oceanography-Methods, v. 6, p. 230-235.

Fellman, J. B., Hood, E., and Spencer, R. G. M., 2010, Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review: Limnology and Oceanography, v. 55, no. 6, p. 2452-2462.

Grasshoff, K., Kremling, K., and Ehrhardt, M., 1999, Methods of Seawater Analysis, New York, Wiley-VCH.

Green, N. W., Perdue, E. M., Aiken, G. R., Butler, K. D., Chen, H. M., Dittmar, T., Niggemann, J., and Stubbins, A., 2014, An intercomparison of three methods for the large-scale isolation of oceanic dissolved organic matter: Marine Chemistry, v. 161, p. 14-19.

Gurtler, B. K., Vetter, T. A., Perdue, E. M., Ingall, E., Koprivnjak, J. F., and Pfromm, P. H., 2008, Combining reverse osmosis and pulsed electrical current electrodialysis for improved recovery of dissolved organic matter from seawater: Journal of Membrane Science, v. 323, no. 2, p. 328-336.

Hansell, D. A., and Carlson, C. A., 2015, Preface, Biogeochemistry of Marine Dissolved Organic Matter: Saint Louis, Academic Press, p. xvii-xviii.

Jaffe, R., Cawley, K. M., and Yamashita, Y., 2014, Applications of Excitation Emission Matrix Fluorescence with Parallel Factor Analysis (EEM-PARAFAC) in Assessing Environmental Dynamics of Natural Dissolved Organic Matter (DOM) in Aquatic Environments: A Review, *in* RosarioOrtiz, F., ed., Advances in the Physicochemical Characterization of Dissolved Organic Matter: Impact on Natural and Engineered Systems, Volume 1160: Washington, Amer Chemical Soc, p. 27-73.

Koprivnjak, J. F., Perdue, E. M., and Pfromm, P. H., 2006, Coupling reverse osmosis with electrodialysis to isolate natural organic matter from fresh waters: Water Research, v. 40, no. 18, p. 3385-3392.

Lindstrand, V., Sundstrom, G., and Jonsson, A. S., 2000, Fouling of electrodialysis membranes by organic substances: Desalination, v. 128, no. 1, p. 91-102.

Mopper, K., Stubbins, A., Ritchie, J. D., Bialk, H. M., and Hatcher, P. G., 2007, Advanced instrumental approaches for characterization of marine dissolved organic matter: Extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy: Chemical Reviews, v. 107, no. 2, p. 419-442.

Pfromm, P. H., Tsai, S. P., and Henry, M. P., 1999, Electrodialysis for bleach effluent recycling in kraft pulp production: Simultaneous control of chloride and other non-process elements: Canadian Journal of Chemical Engineering, v. 77, no. 6, p. 1231-1238.

Repeta, D. J., 2015, Chapter 2 - Chemical Characterization and Cycling of Dissolved Organic Matter, *in* Carlson, D. A. H. A., ed., Biogeochemistry of Marine Dissolved Organic Matter: Saint Louis, Academic Press, p. 21-65.

Ruiz, B., Sistat, P., Huguet, P., Pourcelly, G., Araya-Farias, M., and Bazinet, L., 2007, Application of relaxation periods during electrodialysis of a casein solution: Impact on anion-exchange membrane fouling: Journal of Membrane Science, v. 287, no. 1, p. 41-50.

Stedmon, C. A., and Álvarez-Salgado, X. A., 2011, Shedding Light on a Black Box: UV-Visible Spectroscopic Characterization of Marine Dissolved Organic Matter, *in* Jiao, N., Azam, F., and Sander, S., eds., Microbial Carbon Pump in the Ocean, AAAS, p. 62-63.

Stedmon, C. A., and Bro, R., 2008, Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial: Limnology and Oceanography-Methods, v. 6, p. 572-579.

Stubbins, A., Lapierre, J. F., Berggren, M., Prairie, Y. T., Dittmar, T., and del Giorgio, P. A., 2014, What's in an EEM? Molecular Signatures Associated with Dissolved Organic Fluorescence in Boreal Canada: Environmental Science & Technology, v. 48, no. 18, p. 10598-10606.

Verity, P. G., 2002, A decade of change in the Skidaway River estuary. I. Hydrography and nutrients: Estuaries, v. 25, no. 5, p. 944-960.

Vetter, T. A., Perdue, E. M., Ingall, E., Koprivnjak, J. F., and Pfromm, P. H., 2007, Combining reverse osmosis and electrodialysis for more complete recovery of dissolved organic matter from seawater: Separation and Purification Technology, v. 56, no. 3, p. 383-387.

Young, C. L., and Ingall, E. D., 2010, Marine Dissolved Organic Phosphorus Composition: Insights from Samples Recovered Using Combined Electrodialysis/Reverse Osmosis: Aquatic Geochemistry, v. 16, no. 4, p. 563-574.